System Biosciences

ExoQuick-TC™

Exosome
isolation from
Media and Urine

ExoQuick-TC™ Exosome Precipitation Solution

Cat. # EXOTCxxA-1

User Manual

Store kit at +25°C on receipt

A limited-use label license covers this product. By use of this product, you accept the terms and conditions outlined in the Licensing and Warranty Statement contained in this user manual.

(ver. 5-2013-02-21)

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List of Components

Item	Catalog #	Reactions
ExoQuick-TC exosome precipitation solution (50 ml)	EXOTC50A-1	50 reactions

The ExoQuick-TC™ kits are shipped at room temperature or on blue ice and should be stored at +25°C upon receipt. Properly stored kits are stable for 1 year from the date received. The reaction size is based on using 5 ml of tissue culture media or urine for exosome isolation. Examples of precipitating exosomes from various Biofluids can be seen in the Table below. For best recovery for both RNA and Protein analysis, we recommend starting with 10 ml sample.

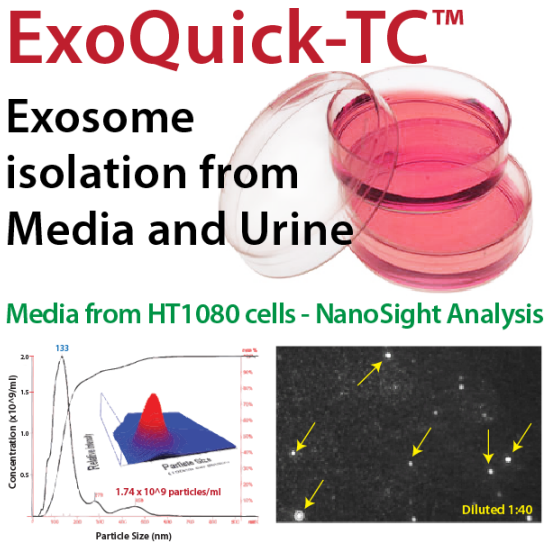
Biofluid	Sample volume	ExoQuick-TC volume
Urine	5 ml	1 ml
Spinal fluid	5 ml	1 ml
Culture media	5 ml	1 ml
For best RNA and Protein recovery (10ml sample)		
Urine	10 ml	2 ml
Spinal fluid	10 ml	2 ml
Culture media	10 ml	2 ml

ExoQuick-TC Exosome Precipitation

I. Overview

Exosomes are small membrane vesicles secreted by most cell types in vivo and in vitro. Exosomes are found in blood, urine, amniotic fluid, malignant ascite fluids and contain distinct subsets of microRNAs and proteins depending upon the tissue from which they are secreted. SBI's ExoQuick-TC exosome precipitation reagent makes microRNA and protein biomarker discoveries simple, reliable and quantitative. Enrich for exosomal microRNAs with ExoQuick-TC™ and accurately profile them using SBI's SeraMir™ qPCR arrays. Downstream protein analysis is also possible with SBI's exosome specific antibodies and ELISA kits.

* No time-consuming ultracentrifugation
* Less expensive than costly antibodies and beads
* More effective than any other method
* Use as little as 5 ml media or urine samples



II. PROTOCOL

A. Exosome Precipitation – 10 ml starting sample

Isolate exosomes with ExoQuick-TC

1. Collect biofluid and centrifuge at 3000 × *g* for 15 minutes to remove cells and cell debris
2. Transfer supernatant to a sterile vessel and add the appropriate volume of ExoQuick-TC Exosome Precipitation Solution to the Biofluid. Some examples are shown in the Table below. Mix well by inverting or flicking the tube

Incubation Time	Biofluid	Sample volume	ExoQuick-TC volume
12 hours-Overnight	**Urine**	**10 ml**	**2 ml**
12 hours-Overnight	**Culture media**	**10 ml**	**2 ml**

3. Refrigerate overnight (at least 12 hours). The tubes do not need to be rotated during the incubation period
4. Centrifuge ExoQuick-TC/biofluid mixture at 1500 × *g* for 30 minutes. Centrifugation may be performed at either room temperature or 4°C with similar results. After centrifugation, the exosomes may appear as a beige or white pellet at the bottom of the tube
5. Aspirate supernatant. Spin down residual ExoQuick-TC solution by centrifugation at 1500 × *g* for 5 minutes. Remove all traces of fluid by aspiration, taking great care not to disturb the precipitated exosomes in pellet
6. Resuspend exosome pellet in 100µl - 500µl of buffer. **Please see the next section of this protocol to determine the appropriate buffer for protein or RNA analysis.**

B. Using Precipitated Exosomes for RNA Extraction

For RNA extraction, we recommend following the protocol outlined in the SeraMir Kit user manual as shown here (Catalog#: RA800A-1, RA805A-1, RA806A-1, RA810A-1, and RA820A-1).

Exosome Isolation and Lysis

1. If frozen, thaw culture media or urine sample on ice
2. Combine 10ml sample + 2ml **ExoQuick-TC**
3. Mix well by inversion three times
4. Place at 4ºC for 6 hours to overnight
5. Centrifuge at 1500 × *g* for 30 minutes
6. Remove supernatant, keep exosome pellet
7. Add 350 µl **LYSIS Buffer** to exosome pellet and vortex 15 seconds
8. Place at room temperature for 5 minutes (to allow complete lysis)
   --- optional--- add 5 µl of SeraMir control RNA spike-in (cat#RA805A-1)

exoRNA Purification

9. Add 200µl of 100% **Ethanol**, vortex 10 seconds
10. Assemble spin column and collection tube
11. Transfer all (600µl) to spin column
12. Centrifuge at 13,000 rpm for 1 minute (check to see that all flowed through, otherwise spin longer)
13. Discard flow-through and place spin column back into collection tube
14. Add 400µl **WASH Buffer**
15. Centrifuge at 13,000 rpm for 1 minute

16. Repeat steps 13 to 15 once again (total of 2 Washes)
17. Discard flow-through and centrifuge at 13,000 rpm for 2 minutes to dry (IMPORTANT !)

exoRNA Elution

18. Discard collection tube and assemble spin column with a fresh, RNase-free 1.5ml elution tube (not provided)
19. Add 30µl **ELUTION Buffer** directly to membrane in spin column
20. Centrifuge at 2,000 rpm for 2 minutes (loads buffer in membrane)
21. Increase speed to 13,000 rpm and centrifuge for 1 minute (elutes exoRNAs)
22. You should have recovered 30-40µl exosome RNA

The yield of RNA from isolated exosomes is different depending on the starting biofluid or the type of cells that were grown in culture. Different cell types secrete varying levels of exosomes.

C. Using Precipitated Exosomes for Protein Extraction

ELISA analysis

SBI offers three ELISA kits (Catalog#: ExoELISA-63, ExoELISA-9, ExoELISA-81) for fast and quantitative analysis of well-characterized exosomal protein markers**: CD63, CD9** and **CD81**.

Exosome Isolation and immobilization

1. If frozen, thaw culture media or urine sample on ice
2. Combine 10ml sample + 2ml **ExoQuick-TC**
3. Mix well by inversion three times
4. Place at 4ºC for overnight (at least 12 hours)
5. Centrifuge at 1500 × *g* for 30 minutes
6. Remove supernatant, keep exosome pellet
7. Centrifuge at 1500 × *g* for 5 minutes to remove all traces of fluid (take great care not to disturb the pellet)
8. Add 200 µl **Exosome Binding buffer** to exosome pellet and vortex 15 seconds
9. Incubate at 37 ºC temperature for 20 minutes to liberate exosome proteins
10. Centrifuge at 1500 × *g* for 5 minutes to remove all residual precipitation solution
11. Transfer supernatant to new centrifuge tube on ice
12. Exosome protein is now ready for immobilization onto micro-titer plate

Please refer to the ExoELISA manual for the complete protocol.

Western blot analysis

For Western blotting analysis, we recommend resuspending the exosome pellet in **1XRIPA buffer**[1] with the appropriate protease inhibitor cocktail.

SBI offers a Western blot ExoAb Antibody Sampler Kit (Cat# EXOAB-KIT-1):which includes four exosomal marker antibodies: **CD63, CD9, CD81, HSP70** and a Goat anti-Rabbit IgG HRP conjugated secondary antibody specifically tested for use in exosomal protein analysis.

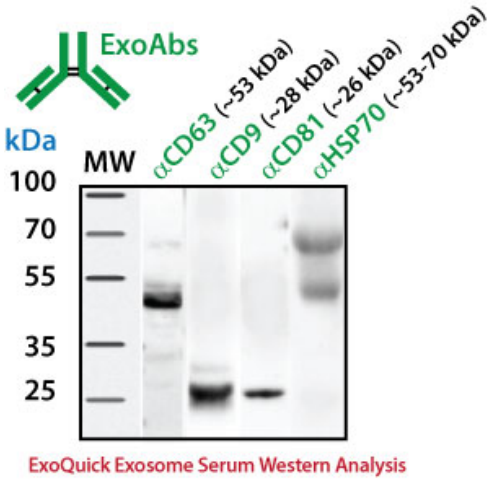
Exosome Isolation and lysis

1. If frozen, thaw culture media or urine sample on ice
2. Combine 10 ml sample + 2 ml **ExoQuick-TC**
3. Mix well by inversion three times
4. Place at 4ºC for overnight (at least 12 hours)
5. Centrifuge at 1500 × *g* for 30 minutes
6. Remove supernatant, keep exosome pellet
7. Centrifuge at 1500 × *g* for 5 minutes to remove all traces of fluid (take great care not to disturb the pellet)
8. Add 200 µl **RIPA buffer**[1] to exosome pellet and vortex 15 seconds
9. Place at room temperature for 5 minutes (to allow complete lysis)
10. Add **Laemmli buffer**[2] (with Beta-mercaptoethanol) and heat at 95ºC for 5 minutes.
11. Chilled on ice for 5 minutes before loading onto gel
12. Perform standard SDS-PAGE electrophoresis and Western transfer onto PVDF membrane
13. Block with 5% dry milk in Tris Buffered Saline + 0.05% Tween (TBS-T) for 1 hour
14. Incubate blot overnight at 4°C with SBI's exosome specific antibody (e.g. CD9) at 1:1000 dilution (5% dry milk in TBS-T)
15. Wash 3X with TBS-T

16. Incubate one hour at room temperature with SBI's Goat anti-Rabbit-HRP antibody at 1:20,000 dilution (5% dry milk in TBS-T)
17. Wash 3X with TBS-T
18. Incubate blot with chemi-luminescence substrate and visualize on film or other imaging equipment

[1] 1X **RIPA buffer** contains:
- 25mM Tris-HCl pH 7.6
- 150mM NaCl
- 1% NP-40
- 1% sodium deoxycholate
- 0.1% SDS

[2] 2X **Laemmli buffer** contains:
- 4% SDS
- 20% glycerol
- 10% 2-mercaptoethanol
- 0.004% bromphenol blue
- 0.125 M Tris-HCl pH 6.8



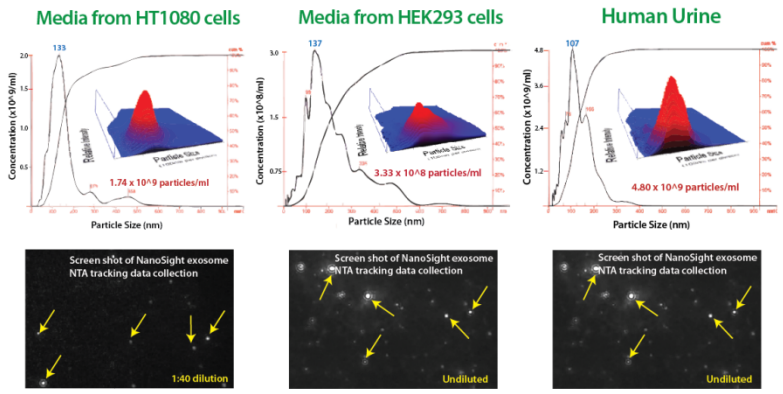
ExoQuick Exosome Serum Western Analysis

III. Sample data and applications

A. NanoSight

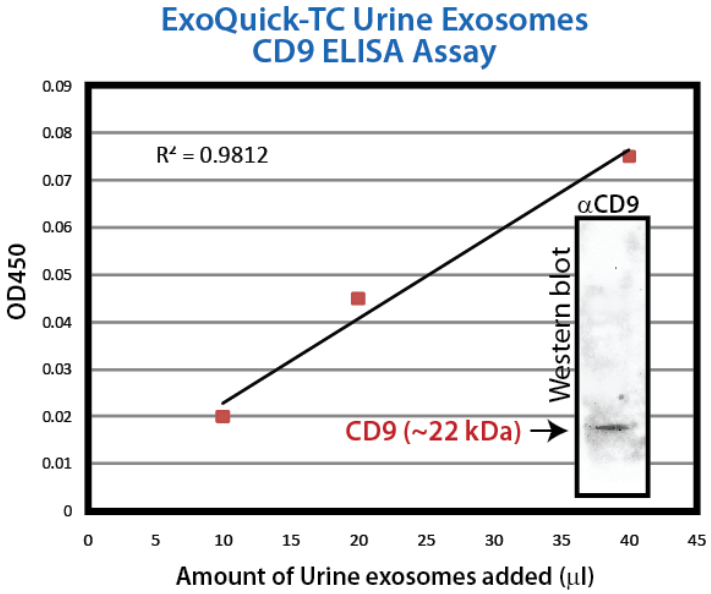
The NanoSight LM10 instrument is based on a conventional optical microscope and uses a laser light source to illuminate nano-scale particles within a 0.3 ml sample introduced to the viewing unit with a disposable syringe. Enhanced by a near perfect black background, particles appear individually as point-scatterers moving under Brownian motion.

The image analysis Nanoparticle Tracking Analysis (NTA) software suite allows users to automatically track and size nanoparticles on an individual basis. Results are displayed as a frequency size distribution graph.



For the NanoSight analysis, 2ml of ExoQuick-TC were combined with 10ml of conditioned media from Human HT1080 lung sarcoma cells or Human embryonic kidney (HEK293) cells. 5ml of normal human urine was combined with 2.5 ml of ExoQuick-TC. All samples were incubated overnight at 4ºC for exosome precipitation. The exosomes were resuspended in 1ml of PBS and visualized on the NanoSight LM10 instrument (The HT1080 culture media were diluted 1:40 and the urine sample diluted to 1:50 prior to analysis). HT1080 culture media analysis showed that ExoQuick-TC isolated 133nm (peak) exosomes with a recovery of 1.74 $X10^9$ particles/ml. The HEK293 showed 137nm exosomes with a recovery of $3.33X10^8$ particles/ml. Normal human urine showed 107nm exosomes with a recovery of $4.8X10^9$ particles/ml. For more information on using the NanoSight instrument for exosome analysis, visit: http://www.nanosight.com.

B. Urine Exosome Marker Protein Analysis

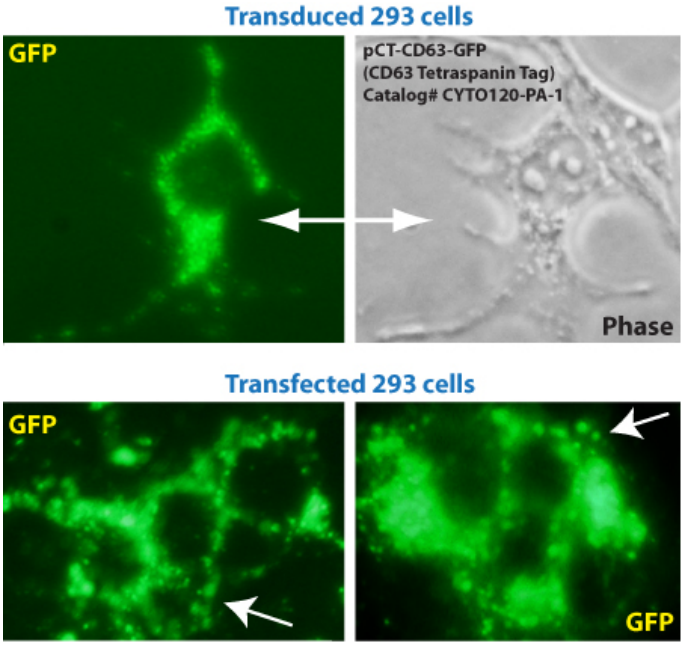


Ten milliliters of normal human urine was combined with 2ml ExoQuick-TC to precipitate urine exosomes. The exosome pellet was resuspended 175 µl buffer and increasing amounts of the exosome suspension was loaded onto an ELISA-ready plate. The CD9 protein was detected using SBI's rabbit anti-CD9 primary antibody and SBI's HRP-conjugated secondary goat anti-rabbit antibody. The size of urine CD9 proteins was determined using Western blot analysis with the same set of antibodies (see inset).

C. Activity Assays: Track Exosomes using Cyto-Tracers

SBI has created a line of lentivector-based Cyto-Tracers™ that utilize GFP-fusion proteins to mark cellular compartments, organelles, vesicles and structures to enable more long-term and more in-depth experimentation. The Cyto-Tracers can be used in transfections as well as packaged into virus to create stable GFP tracer cell lines in primary cells, tumor cell lines and stem cells.

The Tetraspanin CD63 protein is a common biomarker for exosomes. With the pCT-CD63-GFP construct you can make you cells of interest secrete exosomes that glow green for downstream functional delivery studies (Cat. # CYTO120-PA-1).



IV. Citations

As featured in: **Exosome Isolation for Proteomic Analyses and RNA Profiling** Douglas D. Taylor, Wolfgang Zacharias and Cicek Gercel-Taylor, Serum/Plasma Proteomics, Methods in Molecular Biology, 2011, Volume 728, Part 4, 235-246, (PDF) »

Tae Hoon Lee, Esterina D'Asti, Nathalie Magnus, Khalid Al-Nedawi, Brian Meehan and Janusz Rak. Review: Microvesicles as mediators of intercellular communication in cancer—the emerging science of cellular 'debris'. Seminars in Immunopathology DOI: 10.1007/s00281-011-0250-3. (PDF) »

Technical References

Adachi T, Nakanishi M, Otsuka Y, Nishimura K, Hirokawa G, Goto Y, Nonogi H, Iwai N. Plasma microRNA 499 as a biomarker of acute myocardial infarction. Clin Chem. 2010 Jul;56(7):1183-5.

De Smaele E, Ferretti E, Gulino A. MicroRNAs as biomarkers for CNS cancer and other disorders. Brain Res. 2010 Jun 18;1338:100-11.

Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB, Tewari M. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A. 2008 Jul 29;105(30):10513-8.

Laterza OF, Lim L, Garrett-Engele PW, Vlasakova K, Muniappa N, Tanaka WK, Johnson JM, Sina JF, Fare TL, Sistare FD, Glaab WE. Plasma MicroRNAs as sensitive and specific biomarkers of tissue injury. Clin Chem. 2009 Nov;55(11):1977-83.

Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007 Jun;9(6):654-9.

Pegtel DM, Cosmopoulos K, Thorley-Lawson DA, van Eijndhoven MA, Hopmans ES, Lindenberg JL, de Gruijl TD, Wordinger T, Middeldorp JM. Functional delivery of viral miRNAs via exosomes. Proc Natl Acad Sci USA; 2010 Apr 6; 107(14):6328-33.

Mathivanan, S. and Simpson, R.J. ExoCarta: A compendium of exosomal proteins and RNA. Proteomics. 2009.21, 4997-5000.

Thery C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. Nat Rev Immunol. 2009. 8, 581-93.

Michael A, Bajracharya SD, Yuen PS, Zhou H, Star RA, Illei GG, Alevizos I. Exosomes from human saliva as a source of microRNA biomarkers. Oral Dis; 2010 Jan; 16(1):34-8.

Luo SS, Ishibashi O, Ishikawa G, Ishikawa T, Katayama A, Mishima T, Takizawa T, Shigihara T, Goto T, Izumi A, Ohkuchi A, Matsubara S, Takeshita T, Takizawa T. Human villous trophoblasts express and secrete placenta-specific microRNAs into maternal circulation via exosomes. Biol Reprod; 2009 Oct; 81(4):717-29.

Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecol Oncol; 2008 Jul; 110(1):13-21.

Simpson RJ, Lim JW, Moritz RL, Mathivanan S. Exosomes: proteomic insights and diagnostic potential. Expert Rev Proteomics. 2009 Jun;6(3):267-83. Review.

V. Technical Support

For more information about SBI products and to download manuals in PDF format, please visit our web site:

http://www.systembio.com

For additional information or technical assistance, please call or email us at:

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Ordering Information: orders@systembio.com

VI. Licensing and Warranty Statement

Limited Use License

Use of the ExoQuick-TC™ Exosome Precipitation Solution (*i.e.*, the "Product") is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:

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This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

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